AMENDMENTS TO THE SPECIFICATION PURSUANT TO 37 C.F.R. § 1.121

1. Please replace the paragraph, beginning on line 18 of page 17, with the following paragraphs that have been amended as follows:

> ~~Figure 3 shows a schematic of one embodiment of a device to split a nonoliter volume liquid sample and move it using gas from a gas source.~~
> Figure 3A presents a schematic of one embodiment of a device wherein the front of the liquid moves by (but does not enter) a gas-intake pathway (50) that is in fluidic communication with the channel.
> Figure 3B presents a schematic of one embodiment of the device wherein gas is introduced to split off a microdroplet.

2. Please replace the paragraph, beginning on line 20 of page 17, with the following paragraphs that have been amended as follows:

> ~~Figure 4 shows a schematic of one embodiment of a device of the present invention to split, move and stop microdroplets using internal gas pressure generation.~~
> Figure 4A shows a schematic of one embodiment of a device of the present invention to split, move and stop microdroplets using internal gas pressure generation.
> Figure 4B shows a schematic of another embodiment of a device of the present invention to split, move and stop microdroplets using internal gas pressure generation.

3. Please replace the paragraph, beginning on line 27 of page 18, with the following paragraph that has been amended as follows:

> ~~Figure 12 is a schematic of one embodiment~~ Figures 12A-J are schematics for an embodiment for manufacturing a sealable valve of the present invention.

4. Please replace the paragraph, beginning on line 4 of page 19, with the following paragraphs that have been amended as follows:

> ~~Figure 15 is photograph showing simply patterning according to the methods of the present invention to create multiple droplets.~~
> Figure 15A is photograph showing simple patterning according to the methods of the present invention to create multiple droplets.
> Figure 15B is photograph showing simple patterning according to the methods of the present invention to create multiple droplets.
> Figure 15C is photograph showing simple patterning according to the methods of the present invention to create multiple droplets.

5. Please replace the paragraph, beginning on line 6 of page 19, with the following paragraphs that have been amended as follows:

> ~~Figure 16 are schematics and photographs of one embodiment of the device of the present invention utilizing a heater.~~
> Figure 16A presents a photograph of one embodiment of the device of the present invention utilizing a heater.
> Figure 16B presents a photograph of one embodiment of the device of the present invention utilizing a heater.
> Figure 16C presents a photograph of one embodiment of the device of the present invention utilizing a heater.
> Figure 16D presents a photograph of one embodiment of the device of the present invention utilizing a heater.
> Figure 16E presents a photograph of one embodiment of the device of the present invention utilizing a heater.
> Figure 16F presents a schematic of one embodiment of the device of the present invention utilizing a heater.
> Figure 16G presents a schematic of one embodiment of the device of the present invention utilizing a heater.

Figure 16H presents a schematic of one embodiment of the device of the present invention utilizing a heater.
Figure 16I presents a schematic of one embodiment of the device of the present invention utilizing a heater.
Figure 16J presents a schematic of one embodiment of the device of the present invention utilizing a heater.

6. Please replace the paragraph, beginning on line 12 of page 22, with the following paragraph that has been amended as follows:

~~Figure 3~~ Figures 3A and 3B show[[s]] a schematic of one embodiment of a device to split a nonoliter-volume liquid sample and move it using external air, said device having a plurality of hydrophobic regions. Looking at Figure 3A, liquid (shown as solid black) placed at the inlet (20) is drawn in by surface forces and stops in the channel at the liquid-abutting hydrophobic region (40), with overflow handled by an overflow channel and overflow outlet (30). In the embodiment shown in Figure 3A, the front of the liquid moves by (but does not enter) a gas-intake pathway (50) that is in fluidic communication with the channel; the liquid-abutting hydrophobic region (40) causes the liquid to move to a definite location. Gas from a gas source (e.g. air from an external air source and/or pump) can then be injected (Figure 3B, lower arrow) to split a microdroplet of length "L". The volume of the microdroplet split-off (60) is pre-determined and depends on the length "L" and the channel cross-section. To prevent the the pressure of the gas (e.g. air) from acting towards the inlet side, the inlet (20) and overflow ports (30) can be blocked or may be loaded with excess water to increase the resistance to flow.

7. Please replace the paragraph, beginning on line 25 of page 49, with the following paragraph that has been amended as follows:

To eliminate the over-running of the liquid over the patches, an overflow channel was introduced in the design to stop the water running over the hydrophobic patch (such as that shown ~~Figure 3~~ <u>in Figures 3A and 3B</u>). The dimensions of the channels varied in depth and width as before. Water placed at the inlet is drawn in and splits into two streams at the intersection point. The two fronts move with almost equal velocity until the front in the main channel reaches the hydrophobic patch. The front in the main channel stopped at the hydrophobic patch; however, the other front continued to move to accommodate the excess injected water. Using this overflow channel design, one can successfully stop aqueous liquids for the full range of variation in channel dimensions.

8. Please replace the paragraph, beginning on line 6 of page 23, with the following paragraph that has been amended as follows:

In addition to using external air, one can also use internally generated air pressure to split and move drops. ~~Figure 4 shows a schematic~~ <u>Figures 4A and 4B present schematics</u> of one embodiment of a device (110) of the present invention to split (*e.g.* define), move and stop microdroplets using internal gas (e.g. air) pressure generation, said device having a plurality of hydrophobic regions. Looking at Figure 4A, liquid (shown as solid black) placed at the inlet (120) is drawn in by surface forces and stops in the channel at the liquid-abutting hydrophobic region (140), with overflow handled by an overflow channel and overflow outlet (130). In the embodiment shown in Figure 4A, the front of the liquid moves by (but does not enter) a gas-intake pathway (150) that is in fluidic communication with the channel. By heating air trapped inside chambers (180) that are in fluidic communication with the microdroplet transport channel via the gas-intake pathway (150), an increased pressure can be generated. The magnitude of the pressure increase inside a chamber of volume V is related to the increase in temperature and can be estimated by the Ideal Gas relation:

9. Please replace the paragraph, beginning on line 11 of page 41, with the following paragraph that has been amended as follows:

Discrete droplet motion in a micromachined channel structure using thermal gradients is demonstrated in the videorecorded images of ~~Figure 6~~ Figures 6A, 6B, 6C, and 6D. The device consists of a series of aluminum heaters inlaid on a planar silicon dioxide substrate (similar to the structure shown in Figure 2) and bonded by glue to a wet-etched glass channel (20 μm depth, 500 μm width). Liquid samples were manually loaded into the two channels on the left using a micropipette. Heating the left interface of each droplet propels it toward the intersection of the channels. At the intersection, the droplets meet and join to form a single larger droplet. Note that, since the channel cross-section is 20 μm x 500 μm, the volume of each of these droplets can be calculated from their lengths and is approximately 50 nanoliters.

10. Please replace the paragraph, beginning on line 17 of page 30, with the following paragraph that has been amended as follows:

As shown in ~~Figure 7~~ Figures 7A, 7B, and 7C the elements are arrayed as two parallel lanes, each 500μm wide, merging into one lane. The individual heaters consist of paired aluminum wires (5 μm) winding across the 500 μm wide region. The broad metal areas on either side of the elements are bonding locations for connection to external circuitry. The width of the aluminum element is 5 μm. The channel in Figure 7C has identical width and design configurations as the heating element lanes in Figure 7A, and is uniformly etched 500 μm wide and approximately 20 μm deep.

11. Please replace the paragraph, beginning on line 7 of page 46, with the following paragraph that has been amended as follows:

A thermo-pneumatic microvalve is utilized in the test structure. The schematic and process flow of the microvalve is shown in ~~Figure 12~~ Figures 12A-J. A corrugated diaphragm is chosen for its larger deflection and higher sensitivity. The diaphragm

(side length = 1000um, thickness = 3um, boss size length = 500um boss thickness = 10um) has a deflection of 27 um at an applied pressure of 1 atm. This applied pressure is generated by a thermo-pneumatic mechanism, which provides a greater actuation force. A pressure of 1 atm is generated in the cavity between the diaphragm and glass by Freon-11 when it is heated 11°C above room temperature. As set forth in ~~Figure 12~~ Figures 12A-J, ten masks are anticipated to fabricate the microvalve.

12. Please replace the paragraph, beginning on line 6 of page 49, with the following paragraph that has been amended as follows:

The results of Example 10, above, demonstrate that hydrophobic and hydrophilic patterns enable one to define and control the placement of aqueous liquids, and more specifically microdroplets of such liquids, on a substrate surface. ~~Figure 15~~ Figures 15A, 15B, and 15C show[[s]] a simple use of this patterning technique to split a liquid droplet into multiple liquid droplets. A concentric pattern of alternating hydrophobic (dark) and hydrophilic (white) sectors was imparted to a silicon substrate (Figure 15A; the diameter of the circle is 1 cm) using the methods of the present invention as described above. A water drop was placed on the pattern (Figure 15B) and the excess water pulled away using a pipet, resulting in multiple drops separated from each other (Figure 15C).

13. Please replace the paragraph, beginning on line 25 of page 49, with the following paragraph that has been amended as follows:

To eliminate the over-running of the liquid over the patches, an overflow channel was introduced in the design to stop the water running over the hydrophobic patch (such as that shown ~~Figure 3~~ in Figures 3A and 3B). The dimensions of the channels varied in depth and width as before. Water placed at the inlet is drawn in and splits into two streams at the intersection point. The two fronts move with almost equal velocity until the front in the main channel reaches the hydrophobic patch. The front in the main channel stopped at the hydrophobic patch; however, the other front continued to move

to accommodate the excess injected water. Using this overflow channel design, one can successfully stop aqueous liquids for the full range of variation in channel dimensions.

14. Please replace the paragraph, beginning on line 10 of page 50, with the following paragraph that has been amended as follows:

Figures ~~16A-E~~ 16A-J are schematics and photographs of one embodiment of the device (910) of the present invention (in operation) utilizing a heater. ~~Figure 16A~~ Figures 16A and 16F show[[s]] that liquid placed at the inlet (920) stops at the hydrophobic interfaces, and more specifically, stops at the liquid-abutting hydrophobic region (940). The inlet (920) and overflow (930) ports were blocked or heavily loaded with excess liquid to ensure that the pressure generated acts only in the direction away from the inlet holes. The heater resistor (991) was actuated by an applied voltage. The flow of current caused resistive heating and subsequently increases the air temperature in the chamber (980) and, therefore, the pressure. After the pressure builds up to a particular value, a microdrop splits and moves beyond the hydrophobic patch ~~(Figure 16B)~~ (Figures 16B and 16G). The drop keeps moving as long as the heater is kept on; the drop velocity decreases as it moves further away. While it is not intended that the present invention be limited by the mechanism by which this takes place, it is believed that the added volume (the volume by which the drop has moved) brings about a decrease in the pressure.

15. Please replace the paragraph, beginning on line 24 of page 50, with the following paragraph that has been amended as follows:

To stop or block the moving drop at a location, two strategies can be employed. In the first method, the inlet and overflow ports were opened to the atmosphere and the heater was slowly turned off. The temperature inside the chamber falls quickly to around room temperature, thereby reducing the pressure inside the chamber. The

water from the inlet flows into the chamber to relieve the pressure ~~(Figure 16C)~~ (Figures 16C and 16H). In the second method, a hydrophobic vent was placed away from the chamber to the right. As soon as the moving drop goes past the hydrophobic vent ~~(Figure 16D)~~ (Figures 16D and 16I), the drop stops moving farther ~~(Figure 16E)~~ (Figures 16E and 16J). Cooling the chamber to room temperature at this instant will cause air to flow back through the vent to relieve the low pressure in the chamber.